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SUSCEPTIBILITY OF WHITE-TAILED DEER TO EXPERIMENTAL HEARTWATER INFECTIONS

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ABSTRACT: Nine white-tailed deer (*Odocoileus virginianus*) were experimentally infected with *Cowdria ruminantium*, the causal agent of heartwater. All deer developed clinical signs; one was killed, one was treated, and seven died within 2 wk postinoculation. Diagnosis of heartwater was based on clinical signs, postmortem lesions and by microscopic observation of *C. ruminantium* in endothelial cells of brain capillaries of dead animals. *Cowdria ruminantium* was passaged by collecting blood from deer at the height of the febrile response and intravenous inoculation of susceptible deer and goats. Tetracycline was effective in the treatment of heartwater in a deer.

Key words: Heartwater infections, *Cowdria ruminantium*, white-tailed deer, *Odocoileus virginianus*, experimental infections, susceptibility, tetracycline.

INTRODUCTION

Heartwater is a tick-borne septicemic, rickettsial disease of sheep, goats, cattle and certain wild Bovidae and Cervidae. The disease is characterized by high fever, anorexia and nervous signs. Hydropericardium is a common postmortem lesion from which the disease derives its name. The causative agent, *Cowdria ruminantium*, is transmitted by several species of ticks in the genus *Amblyomma*. *Amblyomma variegatum* is indigenous to sub-Saharan Africa and is suspected of having been introduced into the Caribbean region as early as 1830 with cattle from Senegal (Curasson, 1943). Guadeloupe and Antigua were known to be infested before 1900 (Morel, 1966). *Amblyomma variegatum* is known to be well established in Antigua, Dominica, Guadeloupe, La Desirade, Marie Galante, Martinique, Montserrat, Nevis, Puerto Rico, St. Kitts, St. Lucia, St. Maarten/St. Martin, and Vieques. Low numbers of this tick have been found on Anguilla, Barbados, Dominica, Saba, and St. Eustatius (Burridge et al., 1984). The existence of heartwater has been confirmed in Guadeloupe (Perreau et al., 1980), Marie-Galante (Uilenberg et al., 1984), and Antigua (Birnie et al., 1985).

In Africa a number of species of wild Bovidae and Cervidae have been shown to be susceptible to natural or experimental infection with *C. ruminantium*. Both indigenous breeds and exotic game may contact heartwater and die (Uilenberg, 1983). In South Africa, heartwater has been diagnosed in naturally infected imported exotic game such as Indian nylghaie (*Boselaphus tragocamelus*), Barbary sheep (*Ammotragus lervia*), Himalayan tahr (*Hemitragus jemlahicus*), and fallow deer (*Dama dama*) (Young and Basson, 1973); and in Mauritius by Java deer (*Cervus timorensis*) (Poudelet et al., 1982). Hofmeyr (1956) experimentally infected fallow deer, Indian blackbuck (*Antilope cervicapra*) and moufflon (*Ovis musimon*) with *C. ruminantium*.

Amblyomma maculatum, commonly called the gulf-coast tick, is well established in states bordering the Gulf of Mexico and southern states along the Atlantic Coast. This species is usually only found inland for 100 to 200 miles, but is also well established in parts of Oklahoma (Strickland et al., 1981). The larvae and nymphs commonly engorge on ground-inhabiting birds, and small mammals. Cattle, sheep, deer, horses, mules, and dogs are the common hosts of the adults (Strickland et al., 1976). In southern Texas *A. maculatum*

was found on 18% of white-tailed deer examined and was the most common tick encountered (Samuel and Trainer, 1970). Uilenberg (1982) established that *A. maculatum* was capable of transmitting *C. ruminantium* to goats. In light of the frequency with which *A. maculatum* feed on white-tailed deer and the experimental potential of these ticks as vectors of heartwater, a study was carried out to determine the susceptibility of white-tailed deer to experimental *C. ruminantium* infection and to describe the course of the clinical disease and gross lesions.

MATERIALS AND METHODS

The Zeerust strain of *Cowdria ruminantium* was isolated from cattle in 1979 at Zeerust in the Transvaal, South Africa and was received by Dr. G. Uilenberg in The Netherlands in infected ticks (*Amblyomma hebraeum*) from J. D. Bezuidenhout (Jongejan et al., 1980). A frozen *A. hebraeum* stabilate was transferred to our laboratory from Dr. G. Uilenberg, Institute for Tropical and Protozoan Diseases, Faculty of Veterinary Medicine, State University of Utrecht, The Netherlands. Twenty ml of *A. hebraeum* stabilate were inoculated intravenously into a steer. A blood stabilate (S-68) was prepared from the steer by collecting blood from the jugular vein during the period of high fever (41.6 C), mixing it with sufficient heparin and DMSO to make a final concentration of 1% and 6%, respectively, dividing it into 1.8 ml aliquots and freezing the aliquots in liquid nitrogen (LN_2). The viability of the organisms was tested every 3 mo by inoculating 3 ml of blood stabilate intravenously into a goat.

Nine 1-yr-old white-tailed deer of both sexes, originating from North Carolina, were supplied by the Southeastern Cooperative Wildlife Disease Study, Athens, Georgia. Upon arrival at the laboratory, they were divided into groups according to size and housed in three separate biocontainment animal rooms (Dardiri et al., 1966). They were provided with suitable bedding, fresh water and pelleted ration. Daily clinical observations were made and rectal temperatures were recorded. Seven adult goats (1 to 5 yr old) of both sexes and mixed breeds were housed in separate rooms and were maintained in a similar manner to the deer.

Infection in this study was initiated by rapidly thawing three 1.8 ml aliquots of frozen blood stabilate (S-68) that had been stored in LN_2 for 594 days. A goat (925) was injected i.v. with 4.5 ml of this stabilate. When the goat developed a high fever (41.1 C), blood was collected in heparin (1% final concentration) and two additional goats (58, 62) were each inoculated with 6 ml of blood. Blood was collected in 1% heparin from these goats when they developed a high fever (41.1 C), pooled, and two white-tailed deer (109, 110) and a goat (56) were each inoculated with 6 ml of blood.

Following the above procedures the sequence of additional inoculations was: blood from white-tailed deer 109 was inoculated into goat 56 and white-tailed deer 108; blood from white-tailed deer 108 was inoculated into white-tailed deer 107; blood from white-tailed deer 107 was inoculated into goat 59; blood from goat 59 was inoculated into goat 63; blood from goat 63 was inoculated into goat 64 and white-tailed deer 101, 103, 105 and 112.

Samples of cerebral cortex or hippocampus, were collected and used in preparation of crushed brain smears. A minimum of 15 smears were prepared and examined from the brain of each animal. Brain smears were made by placing approximately a 2 mm cube of brain material between two clean glass microscope slides. The tissue was crushed using one slide and then spread thinly over the surface of the other. The smears were air dried, fixed in methanol for 5 min, stained with Giemsa and examined microscopically for the presence of *C. ruminantium*. A diagnosis of heartwater was made when characteristic colonies of *Cowdria* were seen in the capillary endothelial cells (Fig. 1).

One of the white-tailed deer (101) was administered 10 ml of tetracycline (Liquimycin injectable, 50 mg/ml, Pfizer) intramuscularly for 3 consecutive days beginning at 5 DPI. At 2, 4 and 6 wk following the initial inoculations the white-tailed deer was reinoculated intravenously with 6 ml of fresh infected blood from sick goats in concurrent experiments using the same isolate. Susceptible goats were inoculated at the same inoculum to test the infectivity of the blood.

RESULTS

The course of clinical disease was observed in nine white-tailed deer. A summary of the responses of infected white-tailed deer and goats is given in Table 1. Deer 109 was killed because of injury on the first day of febrile response (39.9 C), deer 101 was treated with tetracycline 5 DPI, and the seven other deer died. The

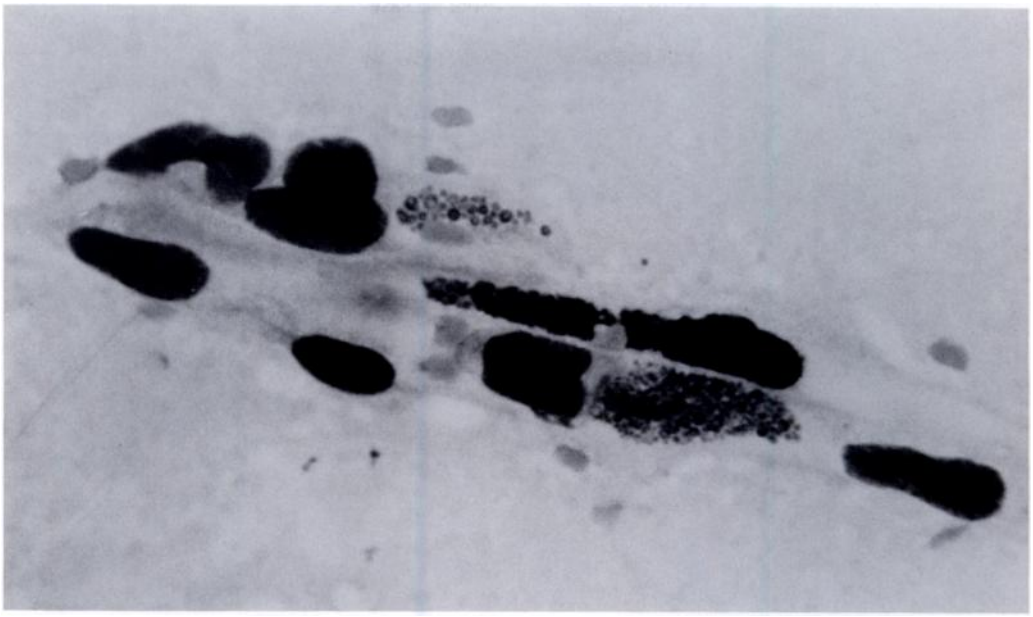

FIGURE 1. A cluster of cerebral cortical capillaries from a goat showing three distinct colonies demonstrating the pleomorphism of *Cowdria ruminantium*. Giemsa, ×1,386.

incubation period was 8 to 11 days with a 2 to 5 day peak febrile response ranging from 39.9 to 40.8 C, and death occurred 11 to 16 DPI (Table 1). The white-tailed deer after the onset of fever were depressed slightly, and less alert than uninfected white-tailed deer. Clinical signs developed rapidly and progressed over a 24-hr period to open mouth breathing, rapid respiration, reluctancy to move, minimal response to visual stimuli, ataxia, proprioceptor defects, saw horse stance, paresis, recumbancy or collapse, tremors, paddling, convulsions and death.

Necropsies were performed on all nine white-tailed deer. Convulsions and paddling resulted in skin abrasions, lacerations, subcutaneous ecchymotic hemorrhage, hematomas and muscle hemorrhage. The most consistent gross lesions were pulmonary congestion, diffuse pulmonary edema and prominent interlobular pulmonary edema. Pleural effusion was observed in only one animal which had 30 ml of clear, straw colored fluid. Pericardial effusion varied from unremarkable to 10 to 30 ml of clear, straw color fluid in five white-tailed deer.

Subepicardial petechial hemorrhage was observed with five white-tailed deer. Severe subendocardial ecchymotic hemorrhages were observed in both right and left ventricles in all the white-tailed deer. The thymuses were congested and had petechial hemorrhage. Mesenteric, prescapular and bronchial lymph nodes were enlarged and congested and had petechial hemorrhages. No diarrhea was evident in any deer. Some hemorrhage in the cecum in one case and petechial hemorrhage along the small and large intestine in another case were consistent with terminal shock. One white-tailed deer suffered a cerebral hemorrhage as a result of a contusion. Changes in other organs were inconsistent.

The clinical signs caused by infection of *C. ruminantium* were observed in eight goats. The incubation period ranged from 6 to 11 days and the peak thermal responses (40.4 C to 41.9 C) lasted 3 to 8 days. All goats developed a fever and six of the eight goats died. The animals that

TABLE 1. Response of white-tailed deer and goats to experimental infection with *Cowdria ruminantium*.

Animal number	Incubation period (days)	Febrile response (days)	Peak febrile response (C)	Days post-inoculation to death	Mortality	Presence of *C. ruminantium*
Deer						
101	ND[b]	0	38.9	ND	Survived[c]	ND[b]
103	10	3	40.2	15	Died	+[a]
105	8	5	40.0	15	Died	+
106	8	4	40.2	13	Died	+
107	8	2	40.4	11	Died	+
108	10	3	40.2	12	Died	+
109	11	1	39.9	12	Euthanatized	+
110	11	2	40.0	15	Died	+
112	8	5	40.8	16	Died	+
Goats						
56	10	3	41.2	14	Died	+
58	9	1	41.8	NT	Survived	NT
59	6	7	41.9	15	Died	+
60	8	5	40.4	12	Died	+
62	9	8	41.9	NT	Survived	NT
63	8	8	41.6	16	Died	+
64	6	5	41.6	11	Died	+
925	11	4	41.8	16	Died	+

[a] Microscopic visualization of *C. ruminantium*.
[b] Not determined.
[c] Treated with Liquimycin starting at 5 DPI.

died developed a thermal response, became progressively more depressed and were anorexic, reluctant to move, two became ataxic and weak, and developed posterior paresis. Four became stuporous and died quietly in sternal or lateral recumbency.

The gross lesions observed in goats were minimal and ranged from slight to severe pulmonary edema. One goat had severe pulmonary edema with prominent interlobular edema and 50 ml of clear straw colored pleural effusion. The goats had 4–10 ml of clear straw colored pericardial fluid, as well as subendocardial hemorrhages.

Colonies of *Cowdria ruminantium* were observed in capillary endothelial cells in Giemsa-stained smears prepared from the brains of all white-tailed deer and goats that died or were killed.

White-tailed deer 101 was treated with tetracycline 5, 6, 7 DPI. This deer did not develop clinical signs of heartwater when inoculated with infected blood at 2, 4 and 6 wk after the initial inoculation. Goats inoculated with blood at the same time intervals all developed heartwater and died.

DISCUSSION

The response of white-tailed deer to infection with *C. ruminantium* was acute and fatal. In general, the incubation period in white-tailed deer was similar to that in goats, but deer developed a more rapid onset of dramatic neurological signs. The most consistent postmortem lesion noted in both deer and goats was pulmonary edema. The postmortem lesions of heartwater in this study were not as dramatic as one would expect judging from the literature. Infection was confirmed by microscopic visualization of *C. ruminantium* in brain smear slides prepared from white-tailed deer and goats which were killed or died.

White-tailed deer are indigenous to the United States and represent an important wildlife resource in many states. The demonstration of susceptibility of white-tailed deer to *C. ruminantium* suggests that they could play a major role in the spread and maintenance of this organism if it were ever introduced into the U.S. The susceptibility of white-tailed deer to *C. ruminantium* and their frequent infestation with the tick *Amblyomma maculatum* (Smith, 1970) could result in great difficulty in the control and eradication of heartwater.

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